

## REMARKS

In an Office Action mailed on February 18, 2004 in the above-identified application, currently pending Claims 1-25 were rejected. Specifically, the Office Action has substantively rejected Claims 1-25 under 35 U.S.C. § 112, second paragraph; Claims 1-3, 8-12 and 17-21 under 35 U.S.C. § 112, first paragraph (written description); Claims 4, 13, 22 under 35 U.S.C. § 112, first paragraph (enablement); and Claims 1, 5, 7, 9, 10, 14, 16, 18, 10, 23, and 25 under 35 U.S.C. §102(b). Each of these objections and rejections is considered separately below. In light of the amendments above and the arguments below, applicants respectfully request reconsideration.

### Claim Objections – 35 U.S.C. §112, Second Paragraph

Claims 1-25 stand rejected under 35 U.S.C. 112, second paragraph, as being indefinite. The Examiner objected to Claims 1 and 10 for not reciting the final step of the method claim; for using the phrase “an effective amount,” which the Examiner considers to be a relative term; and for use of the term “strain” as referring to  $\alpha$ -proteobacteria. In response, applicants have amended herein independent Claims 1 and 10 to introduce the phrase “for controlling crown gall disease on plants” to complete the method claim format. As to the phrase “an effective amount” in Claims 1 and 10, applicants submit that in accordance with the invention, an effective amount is defined as that “amount sufficient to establish sufficient bacterial growth so that infection by crown gall inducing bacteria is inhibited or diminished as compared to infection by crown gall inducing bacteria in the absence of the biocontrol agent.” (See specification, page 8, paragraph [00031]). Therefore, applicants believe that the phrase “an effective amount” is not relative, but is adequately specific to define the metes and bounds of the invention. Furthermore, as suggested by the Examiner, the term “strain” has been deleted as a modifier for  $\alpha$ -proteobacteria. Also, in Claims 4, 13, and 22, reference to the pT2TFXK plasmid has been deleted herein. Applicants submit that no new matter has been added by the addition of these claim amendments.

### Claim Objections – 35 U.S.C. §112; First Paragraph

Claims 1-3, 8-12 and 17-21 stand rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification. In essence the office action asserts that the applicants have claimed a genus of sequences, and allegedly have not

structurally defined the scope of the genus. In response, for clarity reasons, applicants have amended herein independent Claims 1, 10, and 19 to structurally define the  $\alpha$ -proteobacteria of the invention as being genetically engineered to express a *tx* operon that produces trifolitoxin. Accordingly, applicants have canceled dependent Claims 5, 14, and 23. No new matter has been added by these claim amendments. Thus, applicants believe that the introduction of these claim amendments will render this rejection moot.

Furthermore, Claims 4, 13, and 22 stand rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to practice the invention. Specifically, the office action asserts that the specification lacks sufficient evidence that the claimed *Agrobacterium vitis* F2/3 (pT2TFXK), ATCC patent Deposit Designation PTA-2356 was deposited in compliance with the requisite deposit rules 37 CFR 1.801- 1.809. In response, the applicants respectfully submit, through their attorney of record indicated below, over her signature and registration number, that the cell lines of the present application have been deposited with the American Type Culture Collection (ATCC) under the terms of the Budapest Treaty, and that the cell lines will be irrevocably and without restriction or condition released to the public upon the issuance of a patent and that the cell lines will be replaced should they ever become non-viable. Therefore, for the purpose of completing the USPTO record applicants submit herewith a copy of the Receipt and Viability Statement provided by the ATCC indicating that the Deposit was received on August 8, 2000.

The office action further asserts that Claims 1-25 were also rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement, due to a lack of predictability in the art. The Examiner asserts that the terms " $\alpha$ -proteobacteria" and "plants" in the claims are overly broad in scope. In response, applicants have amended independent Claims 1, 10 and 19, for clarification purposes only, to include those species of  $\alpha$ -proteobacteria which are capable of controlling crown gall disease.

Furthermore, in order to obviate this objection and facilitate prosecution on the merits, applicants submit that Claims 1 and 10 have been amended to recite perennial plant species, which are known to be susceptible to crown gall disease. Specifically, these plant species tend to be long-lived plants, such as grapes, stonefruits and roses.

In addition, applicants submit that indeed taxonomically divergent species of plants have been found that are responsive to the crown gall controlling  $\alpha$ -proteobacteria.

Specifically, recent laboratory experiments conducted by the inventors have shown that trifolitoxin-producing bacteria also prevented crown gall disease on grapes, specifically two cultivars of grapes. The results of these experiments have been summarized in Table 1 herein below. Copies of photographic images illustrating control or inhibition of crown gall disease on a variety of grapes are also enclosed herewith as Exhibits A-F. Table 1 also shows that a non-recombinant trifolitoxin-producing strain, *Rhizobium leguminosarum* bv. trifolii T24, also can reduce crown gall formation. The results shown in Table 1 further illustrate that trifolitoxin production in two different backgrounds is capable of controlling crown gall. Additionally, the results demonstrate the efficacy of the methods of the invention beyond simply *Nicotiana glauca*.

**Table 1**

|               | Phenotype of | Candice | Concord | Concord |
|---------------|--------------|---------|---------|---------|
|               | co-inoculum  | CG78    | CG78    | CG435   |
| Water         | control      | 0       | 0       | 0       |
| F2/5(pT2TFXK) | TFX+         | 100     | 83      | ND      |
| F2/5(pT2TX3K) | TFX-         | 0       | 0       | ND      |
| CE3(pT2TFXK)  | TFX+         | ND      | ND      | 83      |
| CE3(pT2TX3K)  | TFX-         | ND      | ND      | 0       |
| T24           | TFX+         | ND      | ND      | 50      |
| T24::Tn5-1    | TFX-         | ND      | ND      | 0       |

Specifically, Table 1 shows percent control of crown gall disease on two lines of grape plants (Candice or Concord) following inoculation with either of two virulent stains of *Agrobacterium vitis* (CG78 or CG435) by co-inoculation with a strain producing TFX (TFX+) or a non-producing strain (TFX-). Applicants note that ND refers to the phrase not determined. Each data point represents at least eight replicates. These results show that plants beyond *Nicotiana glauca* are susceptible to control of crown gall disease using the methods and compositions of the invention. Therefore, applicants respectfully request that in view of these claim amendments and the additional results presented herein, the rejection should be reconsidered and withdrawn.

#### Claim Rejections – 35 U.S.C. §102

Claims 1, 5, 7, 9, 10, 14, 16, 18, 19, 23, and 25 stand rejected under 35 U.S.C. 102(b) as being anticipated by Robleto, et al. Environmental Microbiology, (1998), Vol. 64, No. 7,

page 2630-2633 alone and in combination with Breil, et al., J. Bacteriol. (1993), vol. 175, pages 3696-3702 and Breil et al., NCBI Accession No. L06719, locus RHMTFXA2G, August 4, 1993.

In response, applicants submit that Robleto et al., and/or Briel et al., do not teach or suggest use of any  $\alpha$ -proteobacteria bearing plasmid pT2TFXK as a method of controlling crown gall disease in plants. These publications are simply directed to DNA sequence analysis, subcloning, insertion mutation analysis of the region required for TFX production/resistance, and showing that addition of TFX production phenotype to a *Rhizobium* strain can increase its nodulation competitiveness. Applicants note that although, Robleto et al., discloses that trifolitoxin production can limit nodulation of trifolitoxin-sensitive *Rhizobium* on bean roots, the disclosure does not teach anyone that trifolitoxin production could inhibit crown gall formation on stems of *Nicotiana*, grape or any other perennial plant species susceptible to crown gall disease.

Furthermore, applicants submit that root nodulation and crown gall formation are induced by closely related bacteria, *Rhizobium* and *Agrobacterium*. The mechanisms of these two processes are biochemically and genetically different. In addition, the root and stem sites of these two processes are also very different. Bacteria in these two habitats face entirely different stresses. As such, the inoculation procedures are also different.

Applicants submit that a skilled artisan would not be able to use the methods described in Robleto et al. (1998) to practice the methods of the invention to control crown gall. Applicants further submit that in Robleto et al. (1998), seeds were inoculated prior to planting and the trifolitoxin-producing strain was forced to compete with thousands of strains for rhizosphere growth and dozens of strains for root nodule formation.

With respect to the Breil et al., only the nucleotide sequence of the trifolitoxin genes is disclosed. Moreover, all of the genes described therein, except *tfxA*, must still be regarded as putative because Breil et al., does not provide any information regarding the function of the genes involved in trifolitoxin production. Essentially all that a skilled artisan would have known is that mutations in the trifolitoxin genes would prevent trifolitoxin production. Therefore, the disclosures of Robleto et al., and Breil et al., either alone or in combination would not be sufficient to suggest or teach one of ordinary skill in the art how to control crown gall disease using the methods of the invention.

Lastly, applicants respectfully acknowledge that Claims 2-4, 8, 11-13, 17, and 20-22

are deemed free from prior art, since the prior art does not teach *Agrobacterium* or *Agrobacterium vitis* F2/5 as bacterial host for trifolitoxin production.

In view of the above claim amendments and remarks, applicants respectfully request that a timely Notice of Allowance be issued in this case.

If any extension of time is required in this or any subsequent response, please consider this to be a petition for the appropriate extension and a request to charge the petition fee to the Deposit Account No. 17-0055. No fee is believed to be due in connection with this response.

However, if any fee is due in this or any subsequent response, please charge the fee to the same Deposit Account No. 17-0055.

Respectfully submitted,



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